

apparent to those skilled in the art in light of the present disclosure. Accordingly, it is intended that all such alternatives, modifications, and variations which fall within the spirit and the scope of the invention be embraced by the defined claims.

The following examples are included to demonstrate preferred embodiments of the invention.

EXAMPLE 1

Construction of p53 Expression Vector

This example describes the construction of p53 expression vectors. The methods used are those commonly known to those skilled in the art. The invention is not limited to any particular expression vector, however.

The adenoviral shuttle plasmids pRSVp53, pRSVpRo, pCMVp53 and pCMVpRo that contain the sense and anti-sense cDNA of p53 are shown in Figure 1. These plasmids were constructed by cloning the 1.7 Kb XbaI p53 cDNA fragment into an adenoviral shuttle vector. Davidson, et al., Experimental Neurobiology 125, 258-267 (1994) is incorporated by reference herein for the purpose of disclosing the preparation of such shuttle vectors. Orientation was determined by restriction digest and confirmed by DNA cycle sequencing. The plasmids were expanded in *E. coli* DH5 α and purified by Qiagen Plasmid Mega/Giga Kits (Qiagen). The purified plasmids were quantified spectrophotometrically with A_{260}/A_{280} values approximately 1.90. Agarose gel (0.8%) electrophoresis confirmed that more than 95% of plasmid DNA was supercoiled.

EXAMPLE 2

Synthesis of Ligand-liposome-DNA Complexes

This example describes one method suitable for the production of the ligand-liposome-therapeutic molecule complex, where the therapeutic molecule is plasmid DNA.

lipids. In this instance, the cationic liposomes will be prepared at a molar ratio of cationic lipid to neutral lipid of about 1:(0.5-3), preferably 1:(1-2).

Transferrin will be mixed with the cationic liposomes and then DNA or other therapeutic molecules. The DNA/Lipid/Tf ratios will be in the range of about 1:(0.1-50):(0.1-100) $\mu\text{g}/\text{nmol}/\mu\text{g}$, preferably about 1:(5-24):(6-36), and more preferably about 1:(6-12):(8-15), respectively.

Another unique feature of the complexes according to the invention is their evenly distributed relatively small size (mean diameter less than about 100 nm, preferably less than about 75 nm, and more preferably about 35-75 nm (50 nm average) diameter). To reach the target tumor, the complexes must be resistant to deegratory agents encountered *in vivo*, and also must be capable of passing through the blood vessel (capillary) walls and into the target tissue. The complexes of the present invention exhibit high resistance to degradation by elements present in serum. The permeable size of the capillaries in tumors is usually 50-75 nm, and the complexes which are less than about 75 nm diameter can pass easily through the capillary wall to reach the target. Based upon transmission electron microscopy, it appears that a unique onion-like layered structure of the LipF-DNA and LipT-DNA complex plays an important role in the small size and, consequently, high transfection efficiency of the complex of the invention observed *in vitro* and, in particular, *in vivo*.

The ligand can be any molecule that will bind to the surface of the target cell, but preferentially to a receptor that is differentially expressed on the target cell. Two particularly preferred ligands are folate and transferrin. The cationic lipid can be any suitable cationic lipid, but dioleoyltrimethylammonium-propane

folate-liposome-wtp53 gene or transferrin-liposome-wtp53 gene, in combination with either radiation or chemotherapy, yielded profound results in studies using a nude mouse model. The high efficiency of this system results in such a high degree of sensitization of JSQ-3 and DU145 human xenograft tumors to radiation that not only is there growth inhibition of the cancer but, in some experiments, the pre-existing tumors and metastases were completely eliminated for an extended period of time. In some instances this period of time (more than one year disease-free) is such that the disease may be considered to be cured. Human breast cancer MDA-MB-435 and human pancreatic cancer PANC I nude mouse xenograft tumors were also shown to be highly sensitized by the systemic administration of either folate-liposome-wtp53 or transferrin-liposome-wtp53 to chemotherapeutic agents including doxorubicin, cisplatin, docetaxel or gemcitabine.

As used herein, the term "transfection" is used to describe the targeted delivery of a therapeutic molecule to eukaryotic cells using the ligand-liposome complex of the invention and entry of the therapeutic molecule into the cell by various methods, such as receptor mediated endocytosis. The target cell may be preferentially selected by the ligand of the complex such that the ligand will bind to a receptor that is differentially expressed on the surface of the target cell.

Preferred pharmaceutical compositions of the invention are those that include, within a pharmacologically acceptable solution or buffer, a complex consisting of a ligand, a cationic-neutral liposome and a therapeutic molecule.

Still further embodiments of the present invention are kits for use in the systemic delivery of a therapeutic molecule by the ligand-liposome complex, as

21